Summer 2008

Progress Toward the Synthesis of Two Novel Fluorophore Appended Cationic Steroid Antibiotics

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Abstract

Attachment of glycine residues to the hydroxyl groups of cholic acid yields potent membrane-active antimicrobial agents termed cationic steroid antibiotics (CSA's). In order to gain insight into the mechanism of biological activity, we have embarked on the preparation of two fluorescently labeled CSA's (**1** and **2**). Presented here is our progress in this endeavor. Specifically, the penultimate intermediate in the preparation of CSA **1** has been prepared from cholic acid in 5 steps and 9% overall yield. Using the same synthetic route, we examined the preparation of CSA **2**. Challenges related to coupling the cholic acid moiety to the fluorophore precluded its synthesis and motivated us to explore new synthetic methodologies to accomplish this end. We are in the process of developing the methodology necessary to append the 8-anilino-1-naphthalenesulfonic acid (ANS) fluorophore to the cholic acid core and prepare CSA **2**.

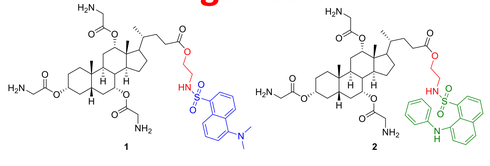
Motivation

- A number of naturally occurring peptide antimicrobial agents have been shown to permeablize the outer-membrane of Gram-negative bacteria making them more vulnerable to hydrophobic antibiotics like erythromycin and novobiocin. Other peptide antibiotics disrupt the membrane so profoundly that they behave as antimicrobial agents themselves.[1]
- Such peptide antibiotics are themselves impractical as potential drugs because they are expensive to produce and unstable in the presence of common proteolytic enzymes.[2]
- Structures that mimic the facial amphipathicity of peptide antibiotics have been developed. One example of such molecules is cationic steroid antibiotics developed in the 1990's.[3] These antibiotics mimic the activity of peptide antibiotics by binding the lipid A component of the outer membrane of Gram-negative bacteria. In fact, certain CSA's have a higher affinity for lipid A than the peptide antibiotics after which they were modeled, specifically polymyxin B.[4]
- Our research involves synthesizing novel fluorescent CSA's. Ultimately, we hope to use fluorescence spectroscopy and kinetics studies to better understand the mechanism of activity of CSA's in various biological models.
- For example, we hope to determine if (1) CSA's have a preference for already disturbed membranes or if they disrupt any membrane with equal potency, (2) how CSA's imbed into the membrane and how many CSA's are needed to destroy a membrane, and (3) how different membrane lipid components affect the binding characteristics of CSA's.[5]

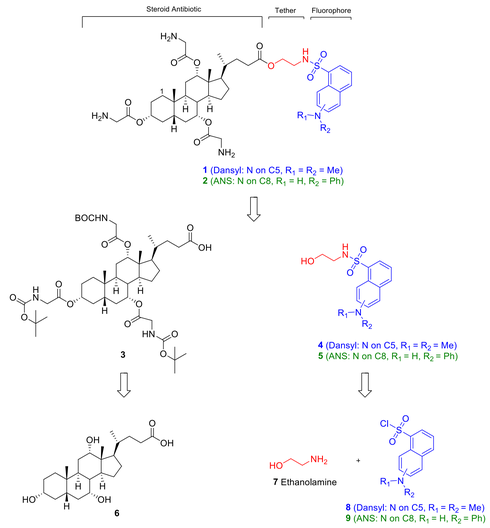
Synthetic Approach

We envisioned the synthesis of the target CSA's by the convergent approach described in the retrosynthetic analysis below. We planned to prepare the final targets (**1** and **2**) by *N,N'*-dicyclohexylcarbodiimide mediated coupling of the modified cholic acid fragment (**3**) and a tethered fluorophore fragment (**4** or **5**).

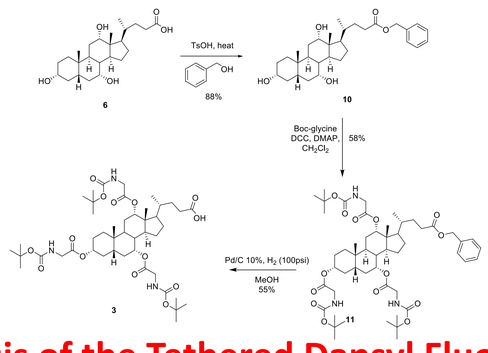
Target CSA's



Retrosynthetic Analysis

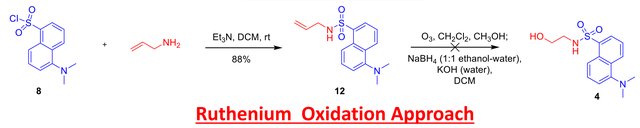


Synthesis of the Cholic Acid Fragment

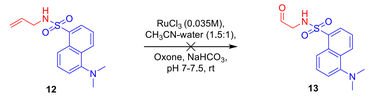


Synthesis of the Tethered Dansyl Fluorophore

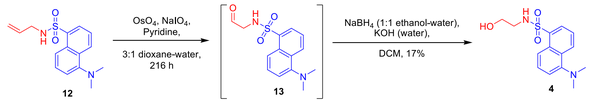
Ozonolysis Approach



Ruthenium Oxidation Approach

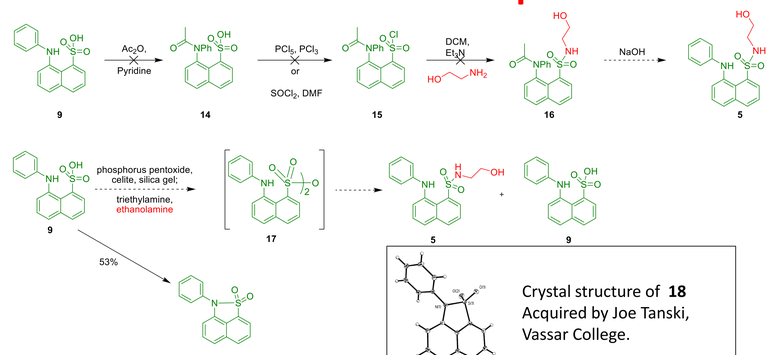


Osmium Dihydroxylation Approach

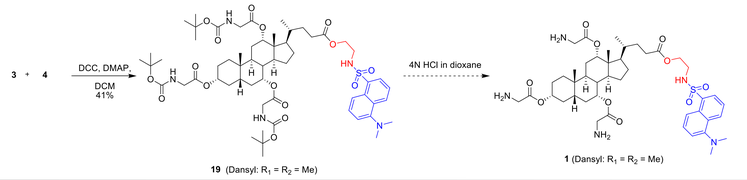


Direct Substitution

Progress Toward the Synthesis of the Tethered ANS Fluorophore



DCC Mediated Coupling and Deprotection



Future Work

The next step in this project will be the deprotection of **19** to give CSA **1**. We will then begin to characterize this compound's biophysical and biological activities. Completion of CSA **2** will require the preparation of the tethered ANS fluorophore (**5**). This effort will begin by examining the reactivity of **18** toward nitrogen nucleophiles.

Acknowledgements

The project was funded by the Kenyon College Research Start-Up Funds, the Drefus Foundation, and a Harvey F. Lodish Development Grant. Additional funding came from the Kenyon College Summer Science Scholars Program 2008. Crystal structures provided by Joe Tanski of Vassar College. Special thanks go to Dr. John Hofferberth for his guidance and patience throughout.

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